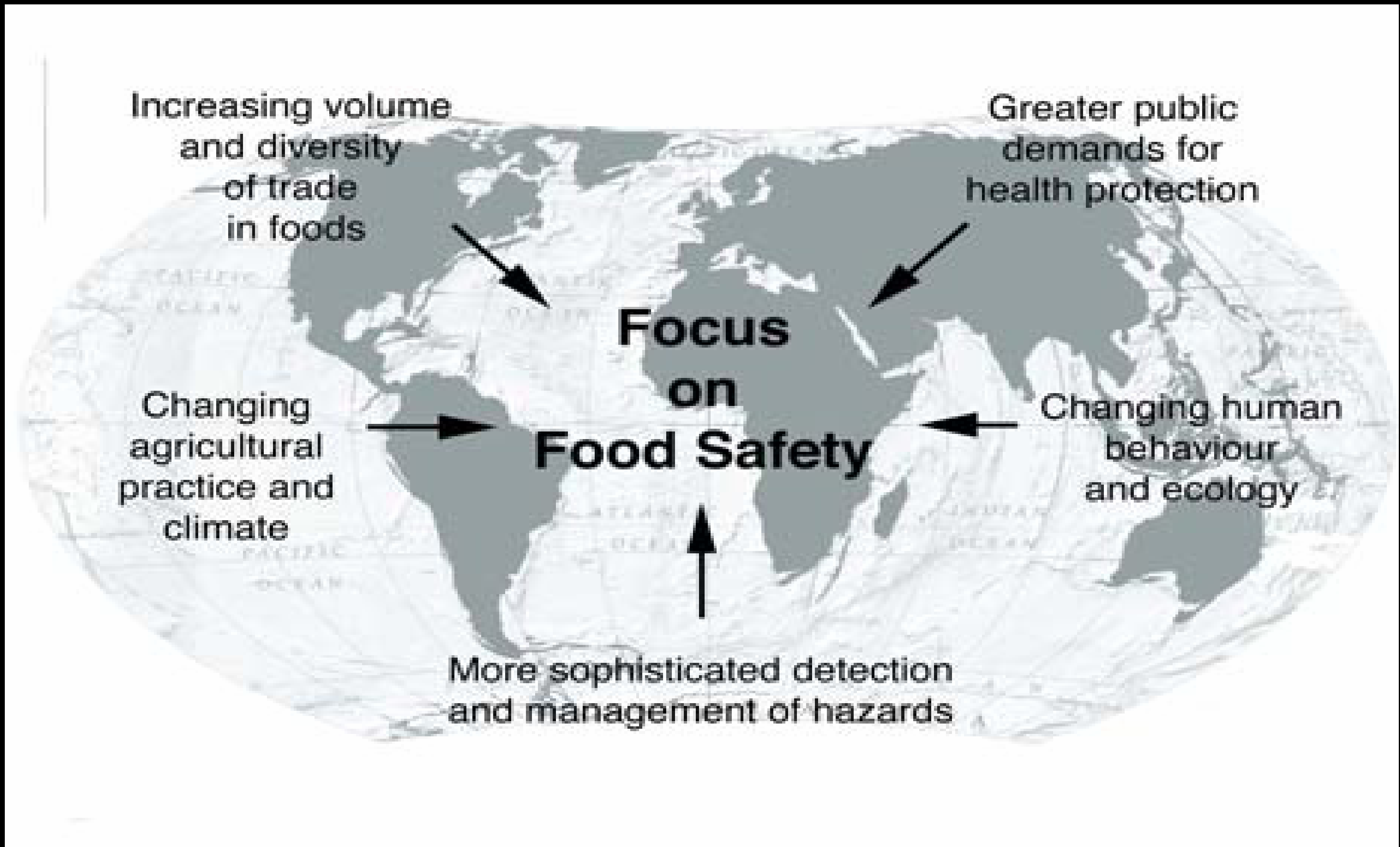
# METHODOLOGY OF RISK ASSESSMENT IN FOODS

VASANTHI SIRUGURI
SCIENTIST - C
&
KALPAGAM POLASA
SCIENTIST- F

**Food & Drug Toxicology Research Centre**
**National Institute of Nutrition (ICMR)**
**Hyderabad 500007**
**kpolasa@yahoo.com**

**Workshop on risk analysis for processed food manufacture in Mumbai on May 15,2010**

# Food safety in the new millennium



# Chemical hazards in the food supply

## Persisting

- **Naturally occurring/biologically derived toxicants:**
  ***Mycotoxins:***
  ***Aflatoxins in milk/milk products, spices, herbal preparations, nuts and oilseeds.***
  ***Ochratoxins in coffee***
  ***Fumonisins in maize and maize products.***

- **Improperly used Agrochemicals:**
  ***Pesticides, veterinary drug residues***

- **Industrial and Environmental pollutants:**
  ***Heavy metals: mercury, cadmium, lead, dioxins***

- **Food Processing chemicals:**
  ***Improperly used food additives and packaging materials***

# Chemical hazards in the food supply

## Emerging

- **Processing induced chemicals: acrylamide in processed foods**
- **Newer adulterants: Melamine in infant foods**
- **New technologies: GM foods, nanoparticles**
- **Climate change and impact on contaminants and chemical residues in food chain.**

# Risk assessment

**Identification and quantification of the risk**
**Resulting from**
**a specific use or occurrence of a chemical,**
**physical or microbiological agent,**
**taking into account**
**possible harmful effects on**
**individual people or society**
**of using the agent**
**in the amount and manner proposed and**
**All the possible routes of exposure**

# Risk assessment process

- 1. Hazard identification
- 2. Hazard characterization
- 3. Exposure assessment
- 4. Risk characterization

**Codex Alimentarius Commission:**

**HAZARD: “a biological, chemical or physical agent with the potential to cause an adverse health effect”**

**Aim of chemical risk assessment :**

**to evaluate whether the chemical has the potential to cause adverse effects in humans based upon review of all available data on toxicity and the biological mechanism that leads to toxicity**

## Hazard identification:

**When chemical hazards arise in the food supply?**

- **Before the raw material enters the food processing**
- **During the storage of raw materials**
- **During the food processing**
- **During the packaging**
- **During the storage of the end-product**
- **During the cooking at home**
- **During its eating**

**Which chemical hazards?:**

- **Industrial and environmental contaminants**
- **Biologically derived contaminants**
- **Contaminants produced during processing**
- **Improperly used agrochemicals**
- **Improperly used additives**

## Data used:

- Human studies (epidemiology, case reports or volunteer studies)

- Toxicity studies conducted in laboratory animals (acute, chronic, reproductive).

- Alternative approaches, including use of in vitro models such as cells cultures or tissue slices, and comparisons with structurally-related chemical substances.

# Chemical hazard identification: Aflatoxin as an example

## Observations

- Shown to induce liver cancer in most animal species studied.

- Epidemiological studies show a correlation between exposure of aflatoxin B1 and increased incidence of liver cancer.

- Aflatoxins metabolised in humans and test animal species to produce an intermediate reactive considered responsible changes in genetic material.

## Hazard identification

***Aflatoxins*** **are considered to cause** ***liver cancer in humans*****, based upon the weight of evidence**

# Hazard characterization

***Qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents***

***Closely linked to hazard identification***

- **Hazard identification: reveals the type(s) of toxicity associated with a particular substance**
- **Hazard characterization: the focus is on the relationship between dose and response that is revealed in these studies and subsequent estimation of dose levels that may cause that response in humans.**

**Issues considered:**

- ***The fate and distribution*** **of the chemical agent in the body**
- ***The action*** **of the chemical agent on tissues or functions in the body.**

# Hazard characterization:

**Critical element: Establishment of dose-response relationship:**

**-prerequisite for characterizing potential risks from exposure to chemicals.**

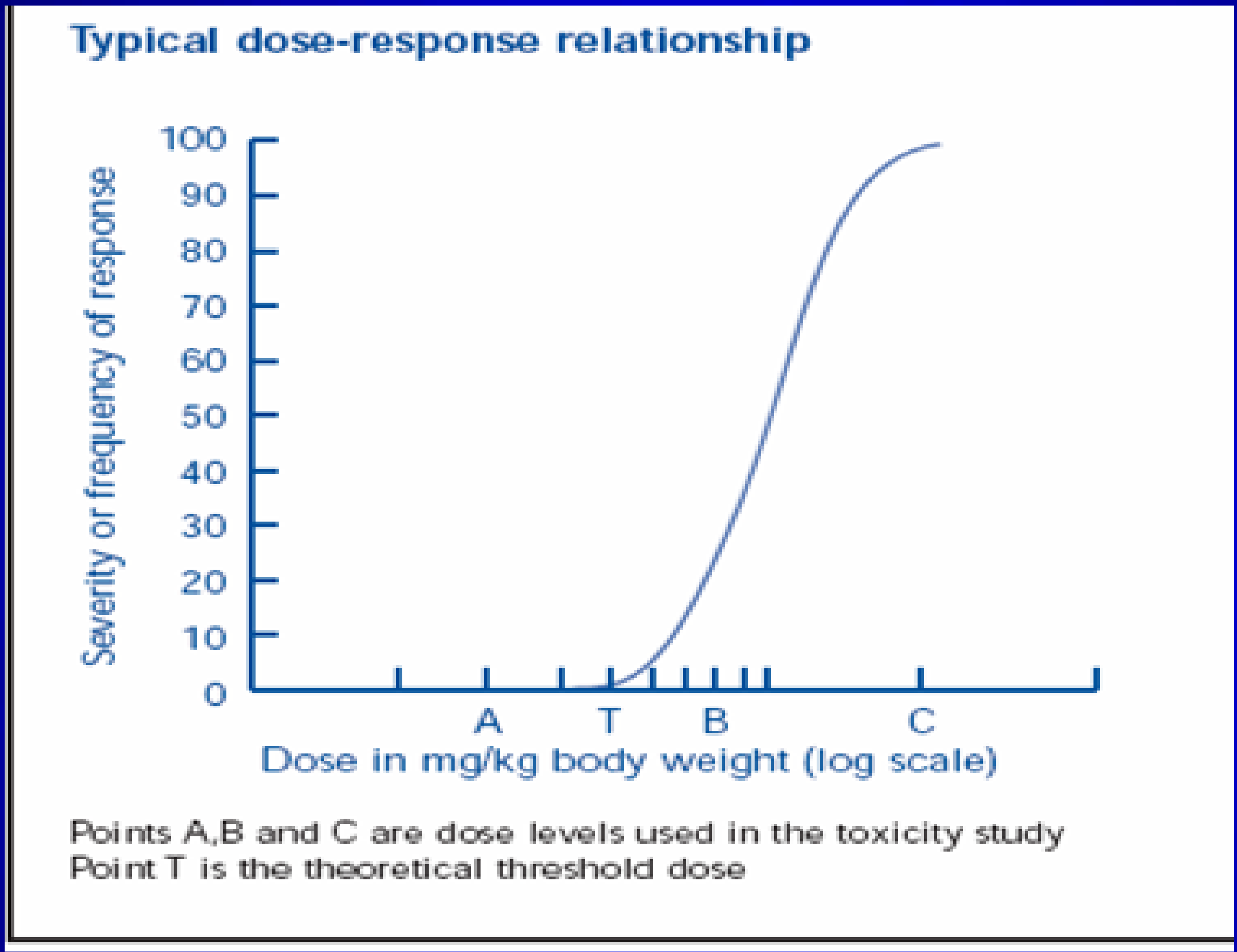
**-starting point for safety evaluation and guideline/standard setting.**

**Risks prediction at given or expected exposure levels based on the dose response curve:**
**For threshold effects (Food additives):**

**No Observed Adverse Effect Level (NOAEL): the highest dose level without adverse effect in the toxicity study that demonstrated the relevant critical effect(s).**

**Lowest Observed adverse effect level (LOAEL)**

**Non-Threshold effects (for genotoxic/ carcinogenic agents): As Low As Reasonably Achievable (ALARA)**




***Reference: Principles of risk assessment of food and drinking water related to human health. ILSI 2001***

## Hazard characterization: Derive Acceptable Daily Intakes (ADI):

An estimate of the amount of a substance in food /drinking water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk

- Assures no hazard to the consumer
- Based on risk assessment and safety factors:
    - Between species 10 x
    - Within species difference 10 x

Overall safety factor, 100 x

# Exposure assessment:

***qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agent via all relevant sources***

**-level ingested determined by the changes in the levels of the agent along the supply chain and after storage and use in the relevant food.**

**-extremely high levels of contamination required to produce an acute adverse response from a single ingestion.**

**-Prolonged intake of a chemical agent at relatively lower levels lead to accumulation within the body, leading to chronic toxicity.**

**-exposure unit of interest is the amount taken repeatedly, potentially over the entire lifespan.**

**-exposure to chemicals is expressed in terms of daily or weekly intake over a lifetime**

## Exposure assessment:

**- Primary source of exposure via ingestion**

**-Degree of exposure determined by the amounts of food and water consumed that contain the chemical, and the levels of chemical contained in those foods.**

***-Intake of a chemical expressed as the amount ingested per unit time (e.g. mg/day). Related to the body weight of an average individual (i.e. mg/kg bodyweight per day). Allows ready comparison of intakes in human populations with the doses used in animal toxicity studies.***

### Information required on:

**1.Type and amount of substances present, in a given food and factors effecting their levels and characteristics**

**2.Amt of food with the chemical substance consumed.**

**3.Conditions and probabilities of consuming high amounts of such food with high levels of chemicals.**

**Dietary exposure assessments:**

**- combine food consumption data with data on the concentration of chemicals in food.**

**-resulting dietary exposure estimate compared with the relevant toxicological reference value for the food chemical of concern.**

**-Assessments undertaken for acute (short-term) or chronic (long-term) exposure.**

**-General equation for both acute and chronic dietary exposure would be expressed as follows:**

$$\text{Dietary exposure} = \frac{\sum (\text{Food chemical conc. X Food consumption})}{\text{Body weight (kg)}}$$

## Approaches for obtaining data on chemical conc.:

- -Maximum levels (Codex MRLs)
- -Reported use levels (from food labels or manufacturers)
- -Monitored levels (raw food or commodities)
- -Levels in foods as consumed (total diet study)

## Approaches for obtaining data on food consumption:

- -Population based methods
- -Household data
- -Individual dietary intake
- -Total diet studies

## Methods for Estimating dietary exposure:

**Screening methods:**

**Dietary exposure above a toxicological reference value (ADI, PTWI)**

**Point estimates/deterministic approaches:**

**$\sum$ Single data point consumption X Single data point chemical conc.**

**Probability distributions:**

**$\sum$ Random food consumption amt from a distribution X Random individual chemical conc. from distribution**

***Calculation repeated thousands of times to give a range of estimated dietary exposures.***

## Risk characterization:

***the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment***

**How likely it is that harm will be done ?**
**How severe the effects will be?**

→ **Risk estimate/ probability of harm at given or expected exposure levels**

## Results of risk characterization:

**1. Comparison of estimated intake or exposure with the acceptable or tolerable level of intake, which is considered not likely to cause harm if repeated daily over an entire lifetime. E.g. Pesticide**

***Intake lower than the ADI – proposed pesticide use acceptable***
***Intake potentially higher than the ADI – consider risk management options***

**Results of risk characterization:**

**2. Estimate of the increased incidence of disease or death that would be associated with different levels exposure repeated daily over an entire lifetime. E.g. Aflatoxins:**

**Review of potency estimates based upon epidemiological evidence**

- ***In absence of hepatitis infection. For every ng aflatoxin consumed per kg bodyweight per day, there will be an*** **estimated additional 0.1 cancers/year/million people**
- ***In presence of hepatitis infection. For every ng aflatoxin consumed per kg bodyweight per day, there will be an*** **estimated additional 3 cancers/year/million people.**

**Potential impact of applying hypothetical standards for permissible levels of aflatoxin in food**

***In areas with low levels of hepatitis infection and low aflatoxin contamination:***

-***Max contamination at 20 µg /kg would give an estimated 0.041 cancers/year/million people, i.e. reducing number by 59 cancers/year per 1000 million people***

***In areas with high levels of hepatitis infection and high aflatoxin contamination***

***- Max contamination at 20 µg /kg would give an estimated 1.7 cancers/year/million people, i.e. reducing number by 1300 cancers/year per 1000 million people***

## International Expert Bodies and Risk assessment

### 1.Joint FAO/WHO Expert Committee on Food Additives (JECFA):

- Food Additives
- Contaminants (CCFAC)
- Residues of Veterinary Drugs in Food (CCRVDF)

### Activity:

- Toxicological evaluation : studies in experimental animals, determination of NOAEL, estimation of ADI/ PTWI/ ALARA, evolving MRLs.
- Setting specification for identity and purity

## 2. Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

- Review of pesticide use patterns
- Toxicological assessment
- Exposure assessment
- Proposes MRLs, based on Good Agricultural Practices (GAP)
- Estimates ADI, based on Toxicological Evaluation
- Compares with ADI, using MRLs and exposure estimates

# Risk Assessment of Nutrients, GMF,GMO & Functional Foods

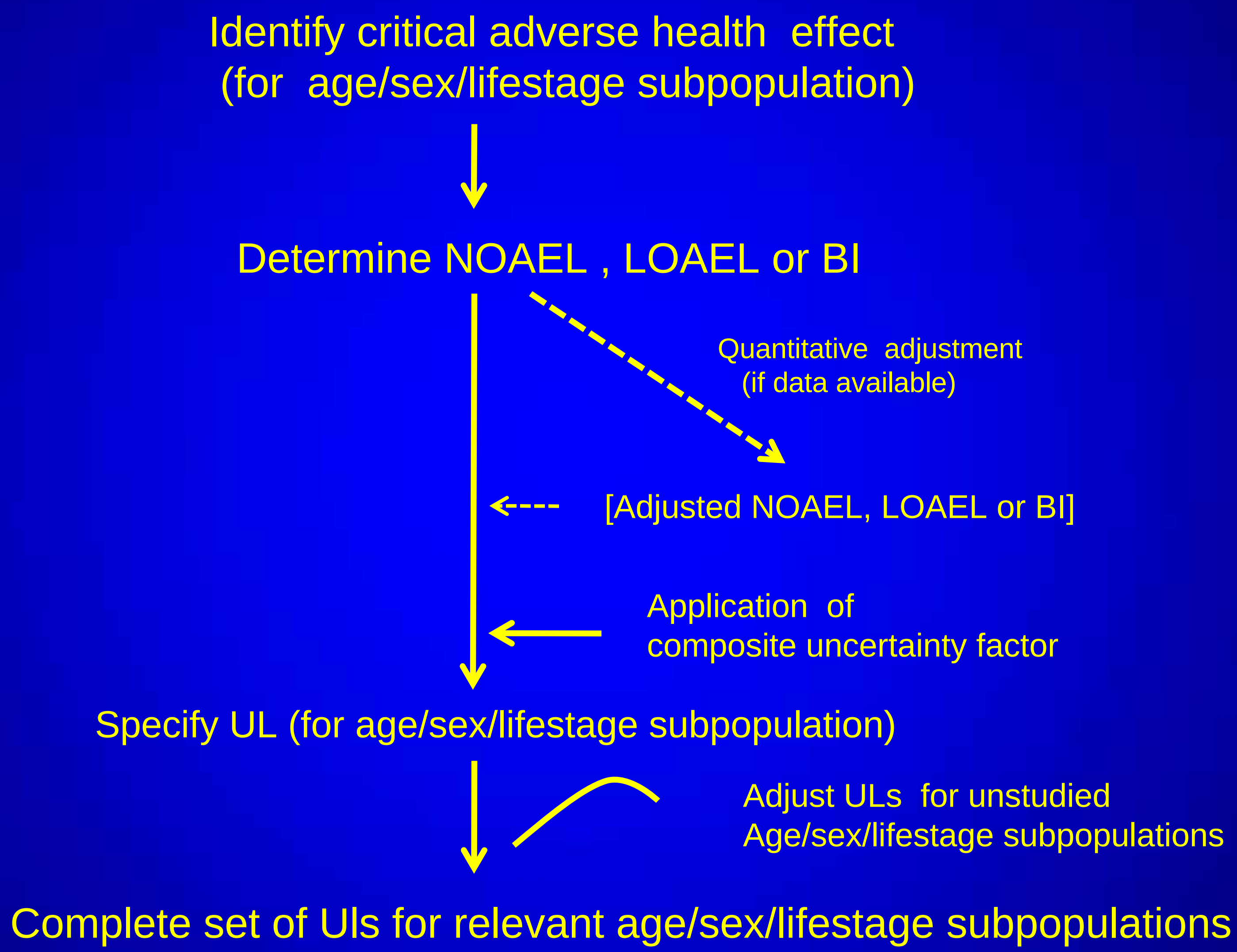
# UPPER SAFE LIMIT OF NUTRIENTS

- Fixing upper safety limit for intake of essential nutrients and it should be applied to functional foods and their bioactive components.
- For eg. Increase in soy consumption can reduce risk of heart disease but exaggerated soy intake may increase risk of tumor proliferation in some individuals.

# STEPS IN RISK ASSESSMENT

- Hazard Identification- scientific review.
- Specify Dose response- establish upper level.(UL) or Bench Mark Index(BMI)
- Intake /Exposure assessment.
- Risk characterization- public health impact.
- Too little nutrients and too much nutrients – both are safety issues.
- Nutrient risk assessments have to be life stage specific eg. adolescents, lactating. Aging populations etc.,

# Steps in Quantifying the Upper Level of Intake




Note : BI = benchmark intake (called benchmark dose in other risk assessment reports);
LOAEL = lowest observed adverse effect level ; NOAEL = no observed adverse effect level; UL = upper level of intake

# NUTRIENT RISK ASSESSMENT

- Regulatory bodies worldwide are considering process for establishing upper level of intake for nutrients.
- Dietary science based supplements, fortified foods, functional foods market is expanding.
- Need for ensuring safety and harmonizing standards internationally
- In traditional approach, regulators provided arbitrary multiple of the intake level known to provide adequate intake of nutrient
- Helps policies for food standards and fortification guidelines.

# Safety Assessment of food produced using a recombinant DNA microbe

1. Description of the recombinant DNA microorganism;
2. Description of the recipient microorganism and its use in food production;
3. Description of donor organism(s);
4. Description of genetic modification(s) including vector and construct;
5. Characterization of genetic modification(s);

Contd.,

## 6. Safety assessment:

a) Compositional analyses of key element;

b) Evaluation of metabolites;

c) Effects of food processing;

d) Expressed substances: assessment of potential toxicity and other traits related to pathogenicity;

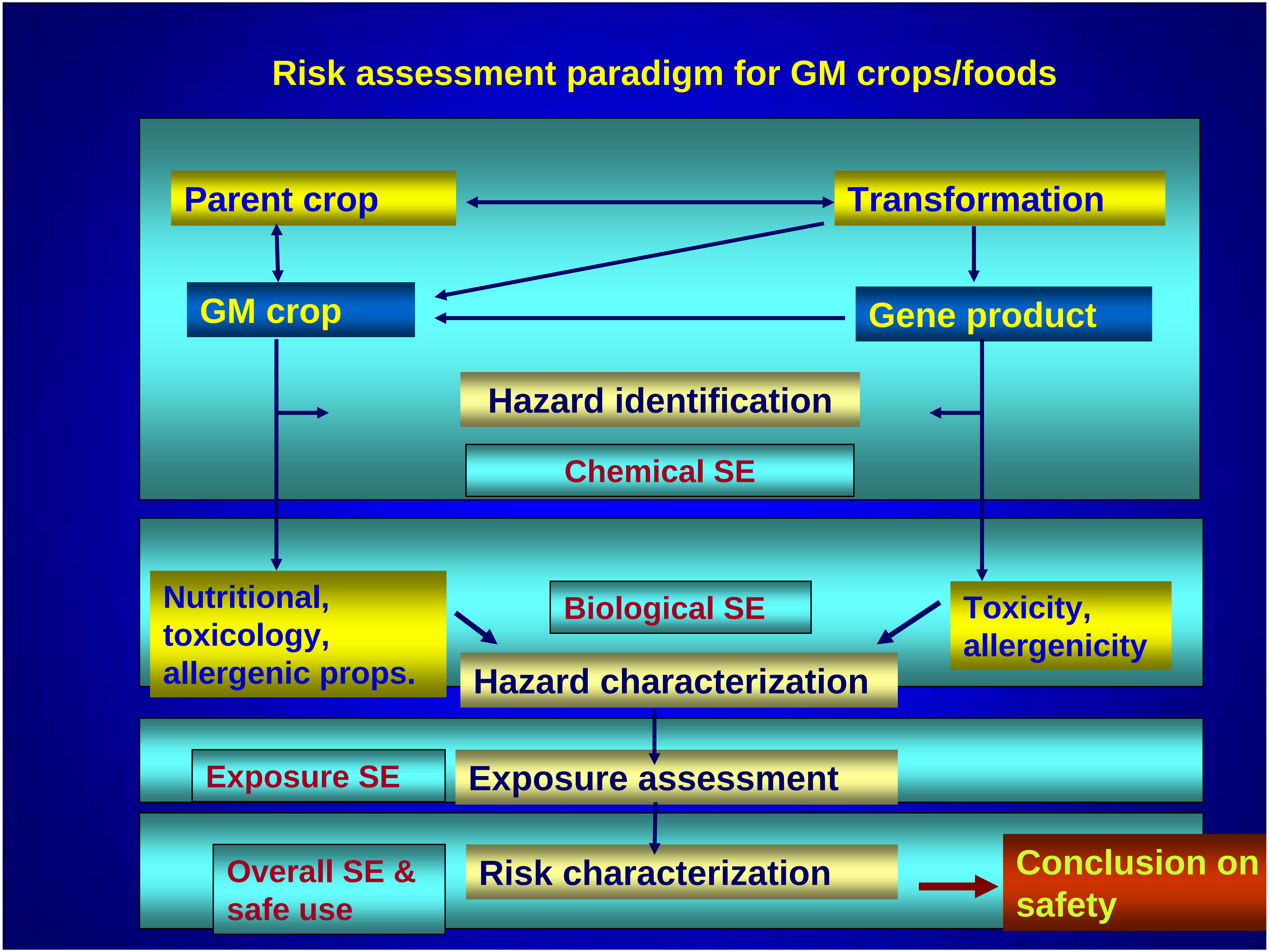
e) Assessment of immunological effects;

- Source of protein
- Amino acid sequence homology
- Pepsin resistance
- Specific serum screening

f) Assessment of viability and residence of micro organisms in the human gastrointestinal tract;

g) Antibiotic resistance & gene transfer;

h) Nutritional modification.


Risk assessment paradigm for GM crops/foods
Parent crop
Transformation
GM crop
Gene product
Hazard identification
Chemical SE
Nutritional, toxicology, allergenic props.
Biological SE
Toxicity, allergenicity
Hazard characterization
Exposure SE
Exposure assessment
Overall SE & safe use
Risk characterization
Conclusion on safety

# FACTORS FOR SUBSTANTIATION OF NUTRITIONAL SAFETY

| Sl.No | Factors |
|---|---|
| 1. | Source and origin of food |
| 2. | Nutrient composition |
| 3. | Presence of anti-nutritional factors |
| 4. | Methods of production and / or preparation |
| 5. | Technical specification including preparation |
| 6. | Purpose to indicate rationale behind the development of functional food |
| 7. | Instruction for storage and use including frequency, dose and duration in relation to dietary recommendations |
| 8. | Interactions with other components of diet and bioavailability |

# FACTORS FOR SUBSTANTIATION OF NUTRITIONAL SAFETY (Contd.)

9. Overall toxicological assessment including toxicokinetics, genotoxicity / intolerance
10. Implications for possible changes in gut micro flora
11. History of safe use
12. Effect on metabolism and physiological functions in human
13. Potential effects on vulnerable groups like infants, elderly, etc.
14. Relation to current dietary recommendations / targets

# FUNCTIONAL FOODS

**Functional foods**

- natural food,
- a food to which a component has been added,
- a food from which a component has been removed,
- a food where the nature of one or more components has been modified,
- a food in which the bioavailability of one or more components has been modified
- or any combination of these possibilities.
- Due to their diversity all functional foods require a case by case evaluation for their safety.
- This process must include both nutritional and toxicological evaluation.

# SAFETY OF FUNCTIONAL FOODS

- Needs to be assessed according to established regulations
- Preclinical test
- Pharmacokinetics
- ADI
- Biomarkers
- Effect biomarkers, biomarkers of exposure
- Modifiers of response by genetic and other environmental factors (susceptibility biomarker) may affect sensitivity of effect biomarkers

CASE STUDY

ANDHARA PRADESH

# TOTAL DIET STUDY

A Report on Dietary Exposure Assessment of Chemical contaminants

*Supported by*

WHO, India Country Office

# OBJECTIVE

To analyse the most commonly consumed foods in Andhra Pradesh for the following selected contaminants after processing as for consumption.

| | | |
|---|---|---|
| Mycotoxins | : | Aflatoxins, T2 Toxin, Fumonisin B1, |
| Pesticide Residues | : | DDT, BHC, Aldrin, Dieldrin, Endosulfan, Monocrotophos, Malthion, Dimethiomate, Methylparathion, Novocran, Dichlrovos |
| Heavy Metals | : | Lead and cadmium |
| Others | : | Fluoride |

# STUDY DESIGN Contd.

The following 22 most commonly consumed foods in Andhra Pradesh as per NNMB 2004-06 Andhra Pradesh rural survey were selected for the study

| | |
|---|---|
| Rice, raw, milled | Banana |
| Jowar | Amaranth |
| Redgram dhal | Spinach |
| Ground nut oil | Chillies dry |
| Buffalo milk | Tamarind |
| Butter milk | Cane sugar |
| Tomato | Egg |
| Brinjal | Chicken |
| Onion | Mutton |
| Potato | Katla (Name of fish) |
| Mango | Bacha (Name of fish) |

The above foods were be analysed for selected contaminants, and the selection of the contaminants was be based on their occurrence in each of the foods.

These belong to one of the food groups as given below:

- Cereals and millets
- Pulses
- Green Leafy Vegetables
- Other vegetables
- Fruits
- Milk and Milk Products
- Fish
- Other Fish Foods
- Spices & Condiments
- Oils and Fat
- Sugar and Confectionary

# FOOD COMPOSITES AND EXPOSURE ASSESSMENT

The amount of food stuff ingested directly decides the amount of contaminant taken, therefore, a percent contribution of all the foods to particular contaminants was assessed. The commonly consumed foods in Andhra Pradesh are very limited. If these foods are grouped as composites, they form 11 form composites.

| S.No. | Food composites | | S.No. | Food Composites | |
|---|---|---|---|---|---|
| 1 | Cereals and Millets | Rice, raw, milled | 6 | Milk and Milk products | Buffalo milk |
| | | Sorghum | | | Buttermilk |
| 2 | Pulses | Red gram Dhal | 7 | Fish | Catla |
| 3 | Green Leafy Vegetables | Amaranth | | | Bacha |
| | | Spinach | 8 | Other Flesh Foods | Meat |
| 4 | Other vegetables | Brinjal | | | Chicken |
| | | Tomato | | | Egg |
| | | Potato | 9 | Spices and condiments | Red chilli Powder |
| | | Onion | | | Tamarind |
| 5 | Fruits | Banana | 10 | Oils and fats | Groundnut oil |
| | | Mango | 11 | Sugars and confectionary | Sugar |

# DIETARY EXPOSURE ASSESSMENT FOR LEAD

- In none of the physiological groups the lead concentration exceeded PTWI of 0.025mg/kg BW.
- The amount of lead consumed varied from 2.7% (13-15yrs) of PTWI to 8.3% (2-4 yrs). If the exposure assessment is carried out at maximum consumption found in the survey , lead seems to be crossing the PTWI in four out of nine physiological groups, 7-9yrs being the highest (1716% of PTWI) and 13-15 year being the lowest (31% ).
- In this study, at the upper consumption levels, there is higher risk in 7-9 years (1716%) which is due to consumption of rice that is highly contaminated as well as the amount of consumption that is high leading to an overall higher exposure to lead. In average consumption levels, pregnant women are having highest consumption (3.6% of ADI) and the age group 13-15 years is at lowest risk (2.7%ADI). These values are lesser than the values in Canadian study in 1998 where about 0.11ug of lead is consumed per day/kgbw and UK population also has a higher intake (0.47 ug/kgbw/day) of lead than Indians where highest value is found in the age group of 1-4 years.

# DIETARY EXPOSURE ASSESSMENT FOR CADMIUM

- The cadmium intake was less than the PTWI of 0.007mg/kgBW in all the physiological groups ranging from 3.5 % (Pregnant women) to 82 % (1-3 yrs). However, in most of the physiological groups it crossed 50% of PTWI,

- At the highest levels of food consumptions cadmium values exceeded the PTWI among 6 physiological groups, pregnant women group being the highest consumer (1847% PTWI) and 4-6 yrs being the group with lowest levels of consumption (11% of PTWI).

# MYCOTOXINS

**Aflatoxins :**

- A total of 24 groundnut oil, 22 chillis and 20 jowar samples were analysed for aflatoxin B1.
- All the samples of ground nut oil, 18 out of 22 chilli, 17 out of 20 jowar were contaminated with aflatoxin B1 at levels ranging from 0.33ug/kg to 50.0ug/kg, 0.1 to 25.0 ug/kg, 0.1 to 1.2ug/kg respectively.
- Sample drawn from Dakkili, Nellore district had the highest concentration (50.0ug/kg).
- A total of 24 buffalo milk samples were analysed for Aflatoxin M1.
- 13 samples out of 24 had a detectable levels of AFM1 with a range from 0.007- 0.09 ug/L.

Out of the three food items which showed the presence of aflatoxins B1 , groundnut oil samples contained highest levels whereas, jowar showed the least.

***Fumonisin B1 and T2 Toxin:***

None of the samples of sorghum were positive for fumonisin B1, while 11 were positive for T2 toxin. The values ranged from1.38- 26.0 ug/kg

**Dietary Exposure Assessment**:

Exposure of AFB1 ranged from 0.08 ug/day in 1-3 yrs to 0.27 ug/day in the age group of 16-17yrs. AFM1 exposure ranged from 0.002-0.004 ug/day. Similarly, the exposure to T2 toxin varied from 0.034micg/day (2-4yrs) to 0.17micg/day (13-15yrs).

Both AFB1 and AFM1 being carcinogens, do not have acceptable daily intake and it is advised that this intake be reduced to as low as possible.

(As Low As Reasonably Possible ALARA or ALARP)

# PESTICIDE RESIDUES

A total of 258 samples of food and 24 samples of water were analysed for various pesticides namely

| | | |
|---|---|---|
| **Pesticides Residues** | **Organochlorines** | **6 isomers of DDT, 4 isomers of BHC, 2 isomers of Endosulfan, 1 derivative (ESS), 2 isomers of Chlordane**<br>**Aldrin, Dieldrin** |
| | Organophosphates | Chlorpyriphos |
| | Synthetic Pyrethroids | Cypermethrin |

None of the samples were free from pesticide contamination. All the samples, including water, were contaminated with one or the other of the 19 pesticides that were analysed. Among all the pesticides, synthetic pyrethroid, Cypermethrin was detected in highest concentration (13.7ug/kg) in rice procured from Ramavaram mandal of East Godavari district.

Higher percentage (>60%) of samples of mango, milk and spinach were contaminated with Cypermethrin compared to all other foods. The second highest concentration detected was that of organochlorine pesticide, Aldrin and organophosphorus pesticide Chloropyriphos in water. Spinach had the highest mean concentration of cypermethrin among all the foods.

Maximum concentrations of hazardous pesticides like $\gamma$ BHC, Aldrin, Dieldrine were detected in water than in food samples. The mean concentration of total DDT was highest (0.843 ug/kg) in rice which was followed by water (0.703ug/kg) and the lowest mean (0.058ug/kg) was detected in potato.

**Dietary Exposure Assessment**

Among the 8 physiological groups, children of 1-3 years and 4-6 years were more at risk to Aldrin, being 6% and 4% of ADI respectively due to the exposure through rice and milk. It was shown in the study that the intake of total DDT is far less than the ADIs in all the age groups ranging from 0.01 – 0.03% of ADIs.

Highest consumption of Chlorpyriphos and total DDT was found in children of age group 1-3 years.

Although fruits like mango and green leaves such as amaranth were contaminated with a large number of pesticides, some even at high levels, but the risk is lower than the other foods as they are consumed in lower quantities.

- The exposure of select contaminants through the food composites has been assessed in select age and physiological groups.
- Cereals & millets food composite is the major contributor of total DDT, Aldrin, Chlorophyriphos, Cypermethrin, Fluoride and Cadmium in all the physiological groups.
- Milk & milk products are the major contributors of gBHC in 4-6 years, 7-9 years and pregnant women groups.
- Equal contributions of, 32% each, milk and milk products and cereals and millets to the gBHC intake in 10-12 years age group children were observed.
- Groundnut oil and milk are the sole contributors of AFB1 and AFM1 respectively, to the diets of all the physiological groups.
- Most of the cadmium in pregnant women's diet comes from green leafy vegetables.
- Milk and milk products are chief contributors of lead to the diets of all the physiological groups.

# Way ahead for food safety chemical risk assessment in India

**1.Set framework to implement successful risk analysis:**

- **A system for providing independent scientific advice and risk analysis/risk assessment to food control authorities.**
- **an operational food safety system and enhance knowledge/awareness about risk analysis for all stakeholders.**
- **Strengthening and networking of National analytical capability including quality assurance programmes/ accreditation.**
- **Formation of a risk assessment consortium between government and industry.**

**2. Develop early awareness, identification and assessment strategies for handling emerging food safety risks.**

**3. Initiate RA method development for 'future exploration'- analysis of scenarios (identification of uncertainties), road mapping (action plans), trend analysis (estimation of future trends-climate change impacts)**


# Thank You